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Do High Levels of C-Reactive Protein in Tanzanian Children Indicate Malaria Morbidity?

N. HURT,[1,2]* T. SMITH,[1,2] T. TEUSCHER,[1] AND M. TANNER[1,2]

Ifakara Centre, Ifakara, Tanzania,[1] and Department of Public Health & Epidemiology, Swiss Tropical Institute, 4002 Basel, Switzerland[2]

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Children under 6 years of age living in an area of Tanzania highly endemic for malaria were tested for C-reactive protein (CRP) in order to determine how the acute-phase response is related to malaria in children of different ages and to investigate whether serum CRP concentrations might be useful in the quantification of morbidity in such children. The median CRP level in the 629 finger-prick blood samples measured, 6.0 mg/liter, was much higher than that reported in the blood of children in Europe. The CRP concentration was correlated with recent illness reported by the parents. High CRP levels were most strongly associated with *Plasmodium falciparum* parasitemia in children under 1 year of age. In older children, lower levels of CRP were associated with parasitemia, and fewer children had increased CRP levels attributable to parasitemia. The levels of malaria-attributable CRP appear to track the acquisition of parasitological and clinical tolerance in this area with very high levels of *P. falciparum* transmission. Determination of CRP levels could be useful in the rapid assessment of the overall burden of morbidity, especially in infants. In areas where malaria is endemic, CRP associated with increased parasite densities provides an objective measure of malaria-specific morbidity. This would be an efficient approach to estimating malaria morbidity risks from small-scale serological surveys.

The acute-phase reactant C-reactive protein (CRP) is a sensitive indicator of the early phase of inflammatory or tissue destruction processes. CRP testing has therefore been advocated for the assessment of general well-being in both human (15) and veterinary (3, 19) screening studies. CRP levels can also provide a simple measure of disease severity (7), the efficacy of therapy (10, 16), and the severity of complications (6). The diagnostic and prognostic significance of CRP has been reviewed (5).

Although CRP is a nonspecific marker of inflammation (4, 17, 23), very high CRP levels are measured during attacks of malaria (8, 11, 18). In the Kilombero district of Tanzania, an area of intense perennial *Plasmodium falciparum* transmission, we found a strong association between CRP levels and malaria parasite densities in pediatric patients at the village health post (11). This suggested that high CRP levels associated with high levels of malaria parasitemia might provide a useful case definition for clinical malaria in areas where asymptomatic parasitemia is common.

Such a definition would be most appropriate in the assessment of morbidity in the community. High CRP levels could represent a more objective definition of morbidity than questionnaire responses and, as an indicator of inflammation, are more sensitive than body temperature measurements (5). However, as an indicator of clinical malaria, its utility in a given setting depends both on the malaria-attributable CRP level being measurable against a background of increased CRP levels from other causes and on the raised CRP levels being clearly associated with clinical malaria and not with asymptomatic parasitemia.

We measured the levels of CRP in finger-prick blood samples collected during field surveys of children in the village where a previous health facility-based study (11) was located. In order to illustrate the burden of morbidity in this community, these levels were compared with those reported from one of the few community-based studies done in western Europe. CRP levels were also compared with the results of health interviews to cross-validate these different measures of morbidity. The relationships between CRP levels, fever episodes, and *P. falciparum* parasitemia were analyzed to determine whether CRP is associated with clinical morbidity or with parasitemia alone. We consider the usefulness of increased CRP levels associated with parasitemia as a case definition in field studies on malaria morbidity in areas where malaria is endemic.

MATERIALS AND METHODS

Study area and study population. The study was undertaken in Namawala, a village in the Kilombero River Plain, Kilombero District (Morogoro Region, Tanzania), which forms part of the Kilombero Malaria Project (12, 26, 30). The Kilombero Malaria Project is being carried out at the Ifakara Centre (Kilombero District, Tanzania) in collaboration with the Tanzanian National Institute of Medical Research by the Swiss Tropical Institute (Basel, Switzerland), Imperial College (London, England), The Universities of Wageningen and Njimegen (Wageningen and Njimegen, The Netherlands), and the Immunology Research and Training Centre, World Health Organization (Geneva, Switzerland). The geographic and demographic aspects of the Kilombero Valley have previously been described in detail (29). Malaria parasitemia is frequent in infants, and the prevalence of *P. falciparum* exceeds 90% in children over 1 year of age. Parasite prevalences and densities show little seasonality (26). Comprehensive data on the health status of children were collected in a nearby village from 1982 to 1984 (28). These investigations revealed high prevalences of protozoan and helminthic infections in children under 5 years of age.

Community-based surveys. Household-based interview and sample surveys were carried out at 2-month intervals between

* Corresponding author. Mailing address: Department of Public Health & Epidemiology, Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland. Phone: (41) 61 284 8428. Fax: (41) 61 271 7951.

March 1989 and August 1992 in Namawala. Material for the type of analyses reported here only came from seven consecutive surveys from February 1991 onward and were obtained from children under 6 years of age. A total of 162 children attended these surveys. At each household visit, the fieldworkers asked the parent or guardian of the child whether the child had suffered from any episode of illness and whether the child had consumed chloroquine during the previous 2 weeks. During the first two surveys, a 24-h recall of fever was also requested.

At each visit, blood was obtained from each respondent by finger-prick, and the blood was collected into a tube (Microtainer Brand Serum Separator tube; Becton Dickinson) and was used to prepare thin and thick blood films. The tubes were transported to the laboratory on ice, and the serum was processed on the same day and cryopreserved. Axillary temperatures were measured with electronic thermometers (Münchner Büro Organisation, International Electronics, Munich, Germany). Fever was defined as any temperature greater than 37.4°C. Febrile children were given immediate treatment, and rapid diagnosis was made by analysis of thick blood films; when appropriate, the children were referred to a health facility.

CRP assays. The concentrations of CRP in serum were quantitated by a radial immundiffusion assay for elevated CRP levels (Human CRP-EL; The Binding Site, Birmingham, United Kingdom). The detection range was 4.0 to 63.4 mg/liter, and concentration intervals of 0.8 mg/liter were discriminated by the assay. The immunoprecipitin ring was examined with a Wild M8 stereomicroscope by using a ×12.5 magnifying eyepiece and a graticule with a scale.

Parasitology. Giemsa-stained thick blood films were screened for the first time in the village to achieve a rapid diagnosis and were reexamined at the laboratory. Parasite densities were calculated by assuming a standard mean leukocyte count of 8,000/μl (25). A blood film was declared negative if no malaria parasite was found per 200 scanned leukocytes, which indicated parasite density of less than 40/μl.

Statistical methods. Many children in the study area had asymptomatic *P. falciparum* parasitemia. A corollary of this is that the presence of parasites does not necessarily mean that malaria accounts for a child's acute-phase response or symptoms and that in such partially immune subjects there is no "gold standard" case definition which can be used to diagnose malaria episodes. We use the term episode to mean a period with a measurable acute-phase response, indicated by a CRP level of ≥6 mg/liter. The existence of a correlation between CRP levels and parasitemia implies that in some episodes, the increased CRP levels are associated with increased levels of parasitemia. The form of the relationship between CRP levels and parasite density can be used to estimate what proportion of episodes with increased CRP levels can be attributed to malaria (for details, see reference 27 and the Appendix). This method does not allow the diagnosis of individual malaria episodes but does provide estimates of the fraction of increased CRP levels attributable to malaria for groups of samples. The use of a conventional case definition (diagnosis of malaria in those children with fevers and parasitemia exceeding a given cutoff) would have resulted in misclassification of a substantial proportion of cases (27).

RESULTS

Levels of CRP in the community. In 51.7% of the samples, CRP concentrations were equal to or greater than 6 mg/liter. The distribution of CRP levels was highly skewed, with 4.9% of the samples having levels exceeding the upper detection limit of the assay (63.4 mg/liter). The median CRP levels in the 629 samples tested are given in Table 1 by age groups. The distribution of total increased CRP levels in children stratified into less than 1 year, 1 to 2 years, and 3 to 5 years of age are presented in Fig. 1. Increased CRP levels were highly prevalent in children in all three age groups, although the median level was lowest in the group of children aged 3 to 5 years (Table 1). The highest proportion of samples with CRP levels greater than 40 mg/liter was found in children less than 1 year of age. Intermediate CRP levels of 20 to 40 mg/liter were most frequently found in children ages 1 to 2 years, resulting in the highest average level found in children in this age group.

TABLE 1. Relationships between CRP levels and age

Age group (yr)	No. of samples	CRP level (mg/liter)[a]		
		q1	Median	q3
<1	58	4.0[b]	7.9	18.3
1–2	231	4.0	9.2	22.7
3–5	340	4.0	4.2	13.5
Overall	629	4.0	6.0	16.6

[a] q1, lower quartile (25th percentile); q3, upper quartile (75th percentile).
[b] Lower limit of detection of the assay.

After adjustment for these age differences, there was a small (Spearman rank correlation, 0.19) but statistically significant correlation between successive CRP measurements for the same child.

Relationship of CRP levels with illness and clinical disease. In order to explore the relationship of perceived illness (reported by the child's parent or guardian) and the child's own condition, proxy morbidity reports and the serum CRP concentrations of these children were compared. In 39% of the questionnaires, the child was reported to have had an episode of illness in the previous 2 weeks. These reports corresponded to significantly higher CRP levels compared with the CRP levels in children without a report of illness in the previous 2 weeks (Table 2).

Only limited data on "nocturnal fevers" were available.

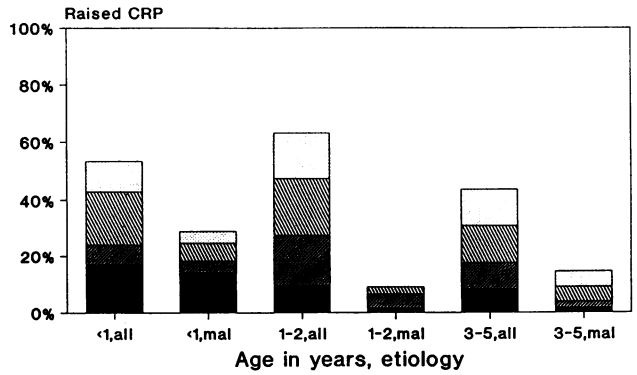

FIG. 1. Distribution of serum CRP concentrations in children aged <1 year (58 samples), 1 to 2 years (231 samples), and 3 to 5 years (340 samples). Bars represent prevalences of increased CRP levels and make up 100% with all CRP levels of <6 mg/liter. All indicates all children with increased CRP levels; mal indicates children with malaria-attributable increased CRP levels. ■, CRP level, >40 mg/liter; ▨, CRP level, 20 to 40 mg/liter; ▧, 10 to 20 mg/liter; ▩, CRP level, 6 to 10 mg/liter.

TABLE 2. Relationships between CRP levels and illness

Symptom or sign	No. of samples	CRP (mg/liter)[a]			Wilcoxon test[b]	
		q1	Median	q3	z	P
Illness in previous 2 wk						
Yes	176	4.0[c]	8.5	22.7	2.93	0.0034
No	278	4.0	4.8	15.0		
Fever reported previous night						
Yes	16	6.7	18.0	44.0	2.50	0.013
No	98	4.0	6.3	15.0		
Temp of >37.4°C						
Yes	27	9.2	23.6	55.2	4.37	0.0001
No	541	4.0	6.0	15.8		

[a] q1, lower quartile (25th percentile); q3, upper quartile (75th percentile).
[b] Nonparametric significance test of CRP levels in positive versus negative respondents.
[c] Lower limit of detection of the assay.

However, it was clear that children whose parents or guardians reported that the child had had a fever during the previous night had higher levels of CRP than those whose parents or guardians reported no such fever.

The CRP levels in children with current temperatures greater than 37.4°C were even higher and were again significantly higher than those in children with lower temperatures. Four of the 27 children febrile at the time of testing had a CRP level of less than 6 mg/liter.

Relationship of morbidity with parasitemia. The overall prevalence of *P. falciparum* parasitemia in the cohort was 87% (Table 3). The prevalence of *Plasmodium malariae* was recorded to be only 1%. *P. falciparum* parasite densities were considerably increased in children with temperatures of >37.4°C, which were measured at their homes, while parasite prevalences in febrile children were similar to those in afebrile children. However, children whose parents or guardian reported an illness during the previous 2 weeks showed a significantly reduced parasite prevalence ($\chi_1^2 = 12.4$; $P < 0.0001$) and also rather lower parasite densities (Wilcoxon test, $z = -1.81$, $P = 0.07$). Chloroquine consumption was reported in 56.1% of the children whose parents or guardians reported morbidity on the questionnaires and in only 5.5% of those whose parents or guardians did not report morbidity. Small reductions in the prevalence and density of *P. falciparum* were also recorded for children who had had fevers the previous night. These differences were not statistically significant.

Relationship of CRP levels with parasite density. Irrespective of whether the child was reported to have been sick in the previous 2 weeks, there was a significant correlation between CRP levels and parasite density (Table 3). Among children with a measured body temperature of less than 37.5°C, there was also a high correlation. However, when the child was clearly currently or very recently ill, the CRP concentration showed little association with parasite density. This is illustrated by the data for children with either a measured fever or whose parents or guardians reported that the child had had a fever the previous night.

Figure 2 presents the prevalence of CRP levels of 6 mg/liter or greater and greater than 40 mg/liter in relation to parasite density and age group. Intermediate patterns were observed with intermediate CRP cutoffs (data not shown).

In the infant group, less than 30% of those with parasite densities of less than 500/μl had a CRP level of 6 mg/liter or greater, and hardly any infants with such low levels of parasitemia had a CRP level of greater than 40 mg/liter. More than 80% of the infants with parasite densities greater than 500/μl had a CRP level of 6 mg/liter or greater, and in many infants (especially those with the greatest parasite densities) the CRP level exceeded 40 mg/liter.

In the oldest age group, there was less of a relationship between parasite density and the probability of having increased CRP levels. More than 40% of the aparasitemic children had a CRP level of 6 mg/liter or greater. However, at high parasite densities the proportion of children with CRP levels above this cutoff was substantially less than that of the infants. The proportion of children in the 3- to 5-year-old age group with CRP levels of greater than 40 mg/liter was small, regardless of the parasite density.

For both CRP cutoffs shown in Fig. 2, the lowest prevalence of increased CRP levels was observed in children with parasite densities of 1 to 500/μl (except in the 1- to 2-year-old age group). The CRP levels in children with a negative result for *P. falciparum* by analysis of the blood film were more likely to be above the cutoff than those in children with positive, but low parasite densities.

TABLE 3. Relationships between *P. falciparum* parasitemia, CRP levels, and illness

Symptom or sign	No. of samples	*P. falciparum* prevalence (%)	*P. falciparum* density/μl[a]			Correlation[b]	P value
			q1	Median	q3		
Illness in previous 2 wk							
Yes	176	78.4	76	1,049	4,764	0.354	<0.0001
No	278	90.3	392	1,226	5,307	0.224	0.0002
Fever reported previous night							
Yes	16	87.5	117	1,190	3,944	0.100	0.72
No	98	93.9	836	2,889	6,890	0.198	0.052
Temp of >37.4°C							
Yes	27	88.9	758	6,370	24,075	0.056	0.79
No	540	87.1	296	1,360	5,179	0.233	<0.0001
Overall	629	87.1	315	1,437	5,550	0.236	0.0001

[a] q1, lower quartile (25th percentile); q3, upper quartile (75th percentile).
[b] Spearman rank correlation coefficient between parasite density and CRP level, controlling for age.

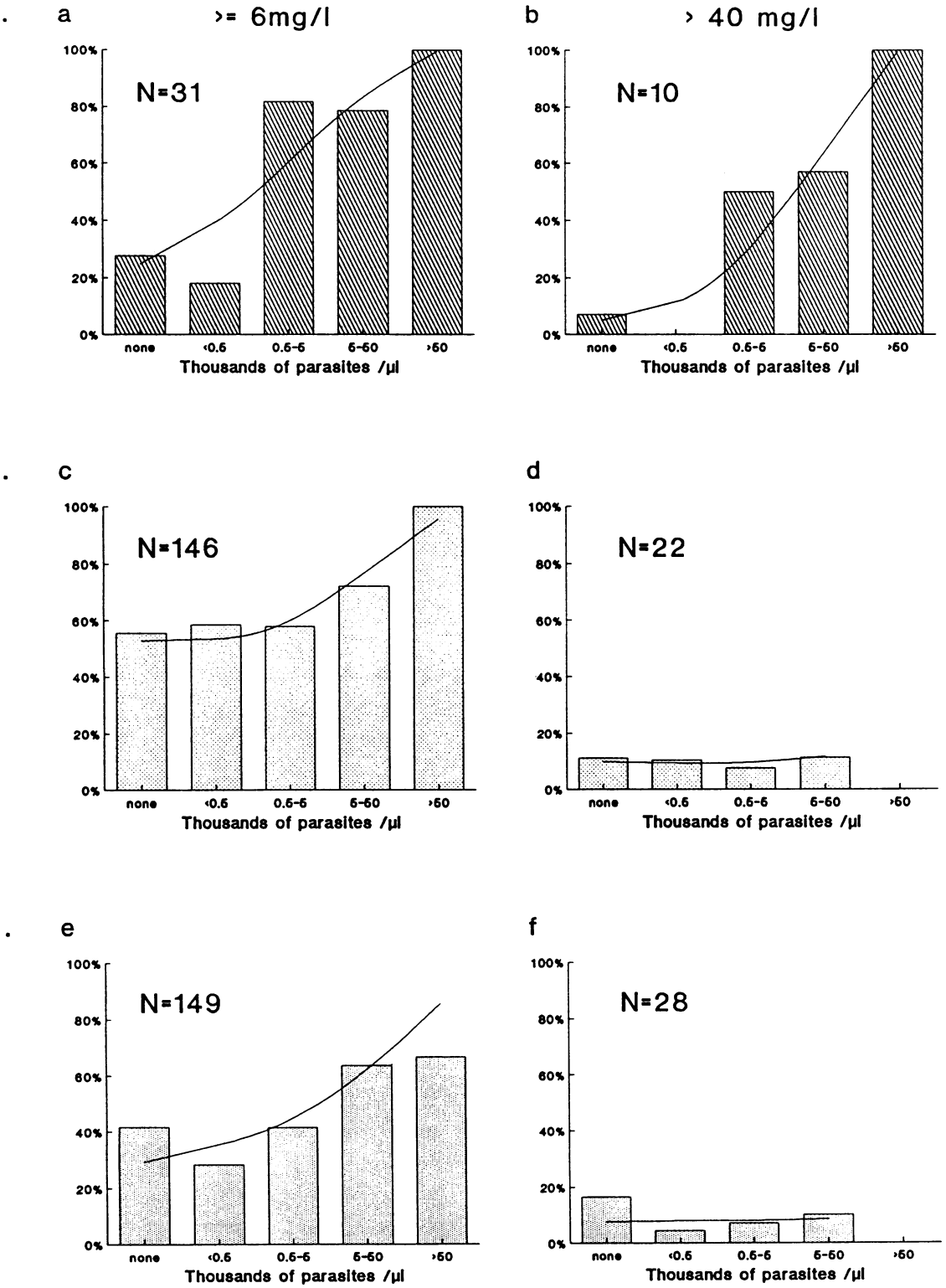

FIG. 2. Prevalence of CRP levels above the cutoff by parasite density (N is the number of samples tested). Bars indicate observed data, and lines indicate fitted regression lines (similar patterns were obtained with intermediate CRP cutoffs). (a) children less than 1 year old with CRP levels of ≥6 mg/liter; (b) children less than 1 year old with CRP levels of >40 mg/liter; (c) children 1 to 2 years of age with CRP levels of ≥6 mg/liter; (d) children 1 to 2 years of age with CRP levels of >40 mg/liter; (e) children 3 to 5 years of age with CRP levels of ≥6 mg/liter; (f) children 3 to 5 years of age with CRP levels of >40 mg/liter.

TABLE 4. Age-specific prevalences of overall and malaria-attributable CRP levels above diagnostic cutoff and corresponding malaria-attributable fractions

Age (yr) and CRP level (mg/liter)	Prevalence (%)		Malaria-attributable fraction
	Overall	Malaria attributable	
<1			
≥6	53.5	28.8	0.538
>40	17.2	14.1	0.820
1–2			
≥6	63.1	9.1	0.144
>40	9.6	1.5	0.156
3–5			
≥6	43.7	14.5	0.332
>40	8.2	1.7	0.207

Figure 2 also shows logistic regression lines fitted to the observed proportions of samples with CRP levels above the given cutoffs. Equivalent lines were fitted for intermediate cutoffs of CRP levels (data not shown).

Attributable fractions of raised CRP levels and distributions of CRP levels. The prevalences of malaria-attributable raised CRP levels in children in the three age groups are also presented in Fig. 1. Table 4 gives the age-specific prevalences of raised CRP levels by using two cutoffs, ≥6 mg/liter for total increased CRP levels and >40 mg/liter for very high CRP levels, respectively, and the malaria-attributable fractions of samples with such raised CRP concentrations. Malaria-attributable increased CRP levels were highly dependent on age. Among the infants, CRP levels of ≥6 mg/liter in sera from 54% of the infants and CRP levels of >40 mg/liter in 82% of the infants were attributable to malaria. Among children in the older age groups, fewer children had raised CRP levels that were attributable to malaria, despite an overall prevalence of increased CRP levels similar to that of the infants. *P. falciparum* parasitemia accounted for about 20% of the CRP levels above 40 mg/liter in these children. Overall, 26.7% of the samples with CRP levels of ≥6 mg/liter could be attributed to malaria parasitemia. The overall proportion of children with malaria-attributable increased CRP levels was therefore 13.8%.

Without carrying out individual diagnoses, it was possible to estimate the distributions of CRP levels in the children with malaria-attributable increases. This was done separately for the three age groups by estimating the numbers of children with malaria-attributable increases in CRP levels for each category of CRP concentrations (see Appendix) (Fig. 3). The estimated distributions of CRP levels in children with nonmalaria episodes are also shown in Fig. 3. These were those children with increased CRP levels which were not attributable to increased parasite densities, but who were not necessarily aparasitemic. Children less than 1 year of age with a malaria episode most frequently had CRP levels of greater than 40 mg/liter, but with increasing age, the modal level of CRP decreased, so that in children in the oldest age group with malaria, CRP levels of 6 to 10 mg/liter were the most frequent.

Children less than 1 year of age with a nonmalaria episode generally had relatively low CRP levels, with CRP levels of 10 to 20 mg/liter being most frequent. There was a gradual increase with age in the relative frequency of high CRP levels in nonmalaria episodes.

DISCUSSION

The biological function of CRP and the regulation of CRP levels are not fully understood. CRP is involved in blood clotting, fibrinolysis, activation of the complement pathway, opsonization of bacteria, and detoxification of foreign materials (13). Interleukins 1 and 6 and tumor necrosis factor alpha stimulate the release of CRP from hepatocytes, leading to a dramatic increase in CRP levels in serum (20). Furthermore, a positive-feedback mechanism of CRP on the blood cells that produce these cytokines has been described (2), and the breakdown products of CRP have been observed to have potent immunomodulating activities (21). In fact, recent findings suggest that CRP, by inducing the interleukin 1 receptor antagonist, has strong anti-inflammatory effects (31). The normal levels of CRP are not well defined, and the increase in CRP levels associated with morbidity varies widely (10). Minor events like physical exercise can result in CRP elevations of up to about 10 mg/liter, suggesting the presence of an inflammatory or tissue destructive process (1).

One of the few community-based studies in Europe measured a median CRP level of 3 mg/liter in healthy young children (15). A total of 20% of the children had CRP levels greater than 12 mg/liter, and 2% had CRP levels greater than 15 mg/liter. Comparison of these CRP levels with those measured in children in Namawala gives an idea of the burden of morbidity suffered by these Tanzanian children (many of whom are apparently healthy). In Namawala, the median CRP concentration was twice that of the western European children, and 27% of the children had CRP concentrations of greater than 15 mg/liter.

Because high CRP levels are associated with objectively measured fevers and current illness, the association between CRP levels and the report of a recent illness provides evidence for the validity of the morbidity questionnaire. The high CRP levels in almost all currently febrile children were expected, since CRP is produced by the mediators of elevated body temperature (20). The four febrile children with CRP levels of less than 6 mg/liter might have been at the beginning of the fever episode, because CRP levels peak at 1 to 2 days after the onset of an episode (14). The known kinetics of CRP also explain why CRP levels were generally higher in children with a morbidity report for the previous night than in those whose episode could have been at any time during the previous 2 weeks (unfortunately, the questionnaire did not elicit the dates of illness episodes recalled by the parent or guardian). The half-life of CRP in plasma is less than 2 days, and elevated CRP levels return to normal within about 1 week (11, 14). Thus, CRP levels can be used to validate the answers of a morbidity questionnaire only if recall periods are within this time interval. For the same reason, the correlation between successive CRP measurements for the same child must reflect interchild variation in either morbidity rate or immunological responsiveness, not the persistence of CRP from one survey to the next.

Reported morbidity was associated with reductions in both malaria parasite density and prevalence. Some of this reduction is associated with the consumption of chloroquine in response to the illness (30). Parasite densities are also reduced during many nonmalarial illnesses because of nonspecific immune responses (11, 22, 27).

Regardless of whether the child's parent or guardian reported an illness episode or not, overall CRP levels and parasite densities were correlated. However, despite this correlation and the overall *P. falciparum* prevalence of 87%, only 13.8% of children had increased CRP levels of 6 mg/liter or greater attributable to malaria. Thus, only a small proportion

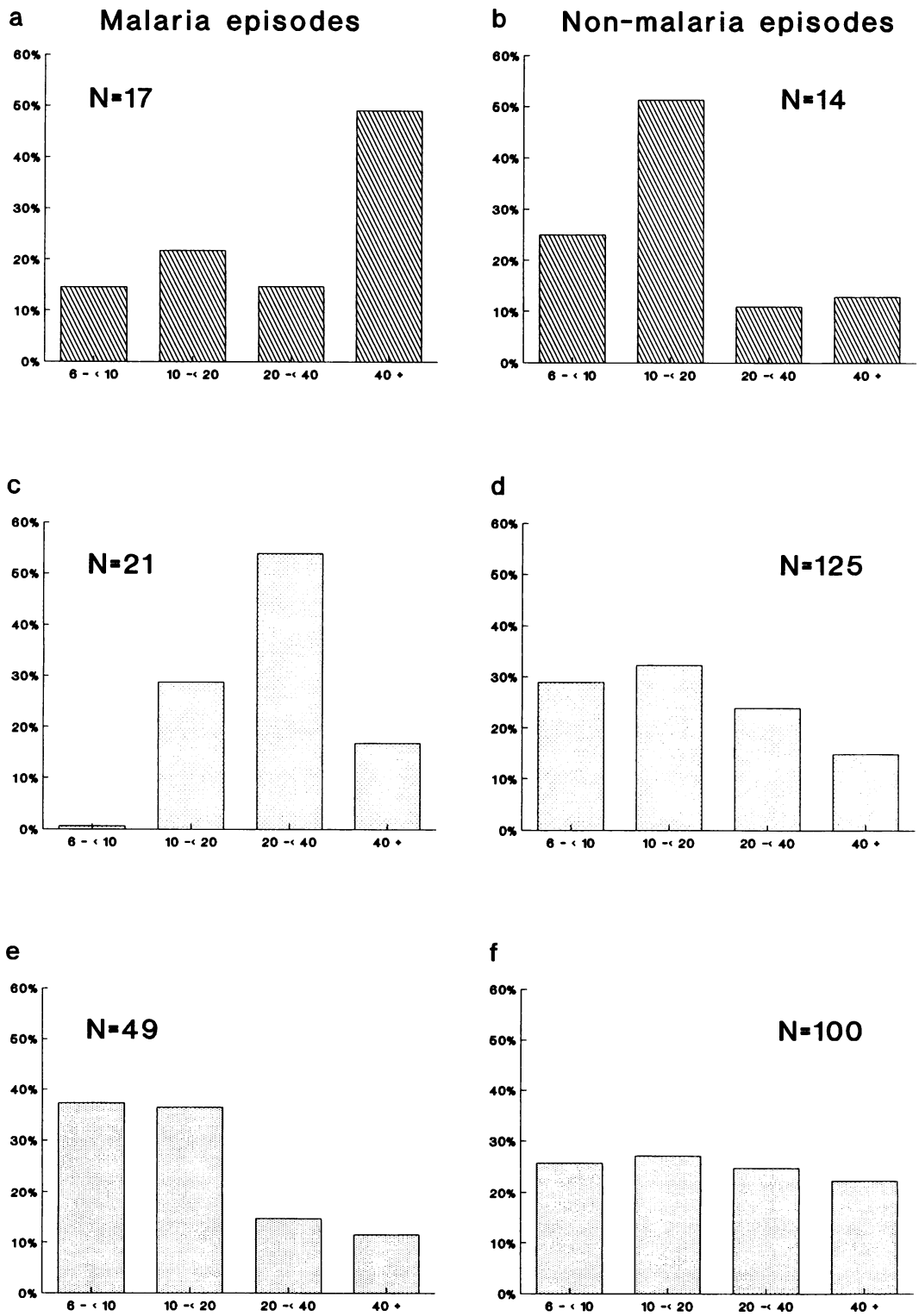

FIG. 3. Estimated distributions of increased CRP levels by etiology (N is the number of samples tested). For attributable fraction estimates, see text and Appendix. (a and b) Children less than 1 year old; (c and d) children 1 to 2 years of age; (e and f) children 3 to 5 years of age.

of the parasitemic children less than 6 years of age had CRP levels of 6 mg/liter or greater.

In infants, malaria seems to be the predominant cause of very high levels of CRP production, accounting for 82% of children with CRP levels of >40 mg/liter. This is in contrast to the situation for older children, who are both less likely to have very high CRP levels and in whom there is also less of an association between high CRP levels and high parasite densi-

ties. The younger children are obviously undergoing fulminant defense reactions against malaria parasites, in contrast to older children with malaria parasites, in whom many of the increased CRP levels in parasitemic children, especially those in the 1- to 2-year-old age group, are not associated with malaria.

CRP could result from many other causes of inflammation prevalent in the area. Different conditions show different age-incidence relationships, and the age dependence of the CRP distributions in children without malaria probably reflects a complex mixture of cause-specific distributions. For instance, respiratory infections are likely to be most common at the end of the first year of life (24), while *Schistosoma haematobium* is more frequent in the older children among those less than 5 years of age (29). Bacterial infections often cause marked increases in CRP levels, while CRP concentration increases during viral fevers are relatively small (4, 17).

The levels of malaria-attributable CRP which we recorded are in accordance with the cause of much, but not all, of the increases being recently evident fevers. In Kilombero, the point prevalence of malaria-attributable measurable fevers (axillary temperature, ≥37.5°C) varies by age to between about 4% in infants and 2% in older children (27). The duration of these fevers is not clear, but we do know that the CRP response is measurable for a much longer period than the period of fever (7, 11). This partly explains why the prevalance of increased CRP levels is much higher than that of measurable fevers.

Asymptomatic malaria infections have many different effects (9). Our results suggest that a small proportion of the CRP production reflects immune activation associated with subclinical infections. This can then explain why high CRP levels were associated with parasite densities even in children reported not to have been sick. This association means that a high CRP level in hyperparasitemic children is not a specific indicator of recent fever caused by malaria. However, CRP levels in parasitemic children were frequently low, so the level of immune activation in response to parasites is heterogeneous even within one age group.

The peak prevalence of very high parasite densities and spells of severe febrile attacks in Namawala is in infancy (26, 27). This is in accordance with the fact that the malaria-attributable fraction of increased CRP levels was highest in the infants. Under constant exposure to *P. falciparum*, immunity develops with age, leading to more moderate parasite-induced cytokine responses and malaria morbidity of less severity. The changes in CRP levels observed in the children with malaria were much as this predicts. Older children with hyperparasitemia were more likely than infants to present with low CRP levels; i.e., the probability of increased CRP levels at a given parasite density changes with age. This indicates a certain degree of tolerance to malaria parasites. It is suggestive that the same CRP level does not have the same clinical significance at different ages.

As a tool in epidemiological studies, CRP is a more sensitive indicator of malaria illness than measured fevers, partly because CRP levels reflect the recent history of fevers as well as current episodes. High CRP levels may also occur in additional clinical malaria episodes in which the symptoms do not include measurable fever. Consequently, CRP could be a good outcome measure in impact assessments of measures aimed at morbidity control. CRP levels represent a more objective measure than questionnaire responses, and this is an advantage in quantitative comparisons between and within communities. CRP levels would also be particularly useful in rapid assessment procedures in which sera are collected anyway but in which the sample size is too small for conventional estimates of malaria morbidity risk made on the basis of measured fevers. Such an approach might prove to be especially valuable in infants, in whom the symptoms of malaria are particularly ill defined and in whom the highest levels of malaria-attributable CRP are observed.

APPENDIX

Statistical methods. The data were stratified according to the age of the child on the day of the survey. For each age group, each sample i was then categorized according to whether the CRP level, c_i, exceeded a given cutoff, C_j. For each sample with parasite density x_i, the risk ratio

$$R_{i,j} = \frac{Pr(c_i > C_j \,|\, x = x_i)}{Pr(c_i > C_j \,|\, x = 0)}$$

was estimated. Pr indicates probability, and $R_{i,j}$ is the risk of the CRP level exceeding the cutoff in a sample with parasite density x_i divided by the risk for an individual in the same age group in the absence of malaria parasites. Previous work (27) suggested that the best estimate of such a relative risk could be obtained by fitting a logistic regression of the form:

$$\log \frac{Pr(c_i > C_j \,|\, x = x_i)}{1 - Pr(c_i > C_j \,|\, x = x_i)} = \beta_{0,j} + \beta_{1,j} x_i^{\tau_j}$$

where $\beta_{0,j}$, $\beta_{1,j}$, and τ_j are the parameters to be estimated (see reference 27). $Pr(c_i > C_j \,|\, x = 0)$ is estimated from the fitted value for $x_i = 0$. This method was therefore used to obtain estimates of $R_{i,j}$. Let N_j be the total number of samples with $c_i > C_j$. λ_j, the fraction of those children with CRP levels greater than cutoff C_j, and where this CRP was attributable to malaria, was then estimated as the mean of the excess risk associated with these N_j different samples, i.e.,

$$\lambda_j = \frac{1}{N_j} \sum_i \frac{R_{i,j} - 1}{R_{i,j}}$$

where the summation is over the N_j samples with $c_i > C_j$ only. The number of samples with a CRP level of $c_i > C_j$ is then $\lambda_j N_j$. By carrying out these computations for a range of different CRP cutoffs, the number of samples with malaria-attributable CRP levels that fell in each range (the distribution of CRP in the malaria-attributable episodes) was estimated. Similarly, the number of children with non-malaria-attributable CRP levels above each cutoff was estimated as $(1 - \lambda_j) N_j$, and hence, the distribution of CRP levels in the children with episodes of etiologies other than malaria was estimated.

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